SUPPLEMENTARY INFORMATION

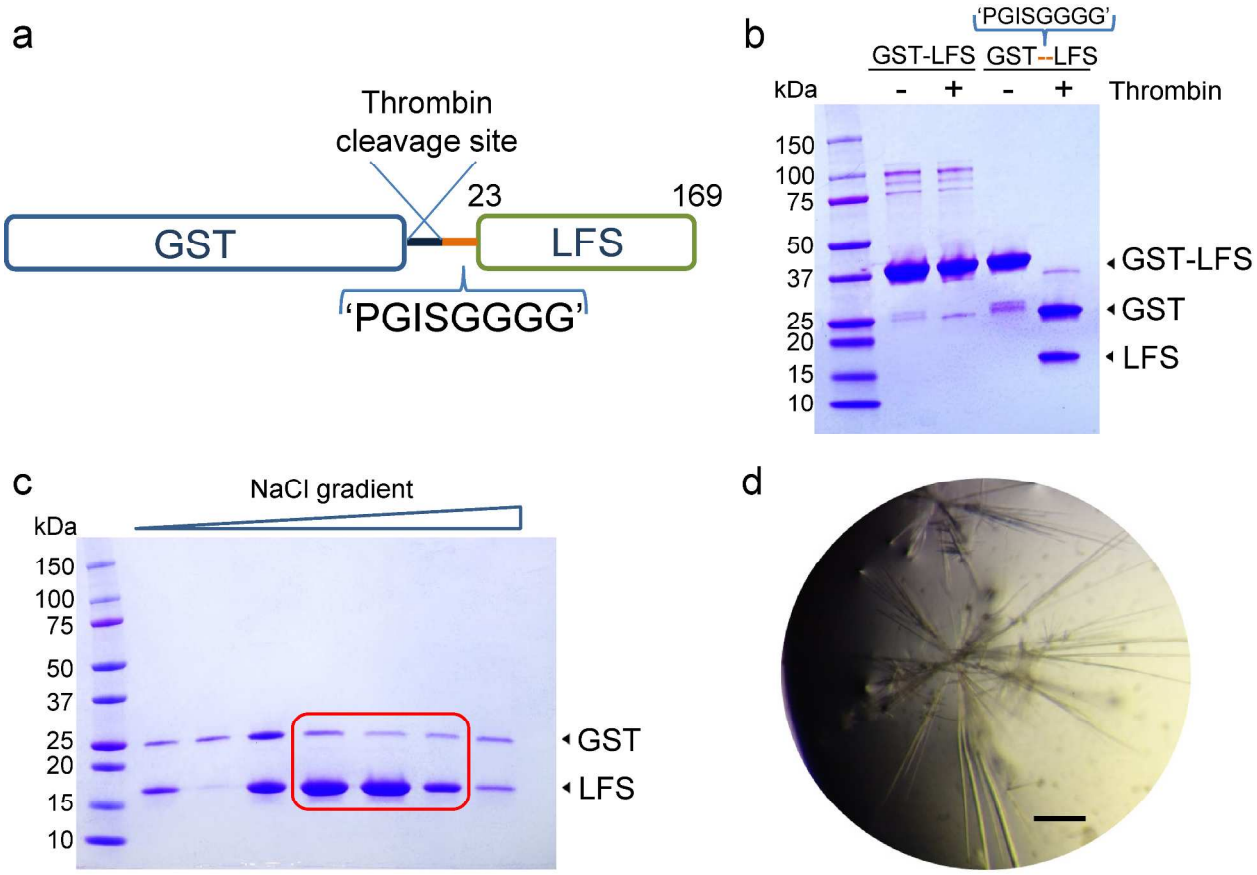
Enzyme that makes you cry – crystal structure of lachrymatory factor synthase from *Allium cepa*

Josie A. Silvaroli[1#], Matthew J. Pleshinger[1,2#], Surajit Banerjee[3,4], Philip D. Kiser[1,5,6], and Marcin Golczak[1,6,*]

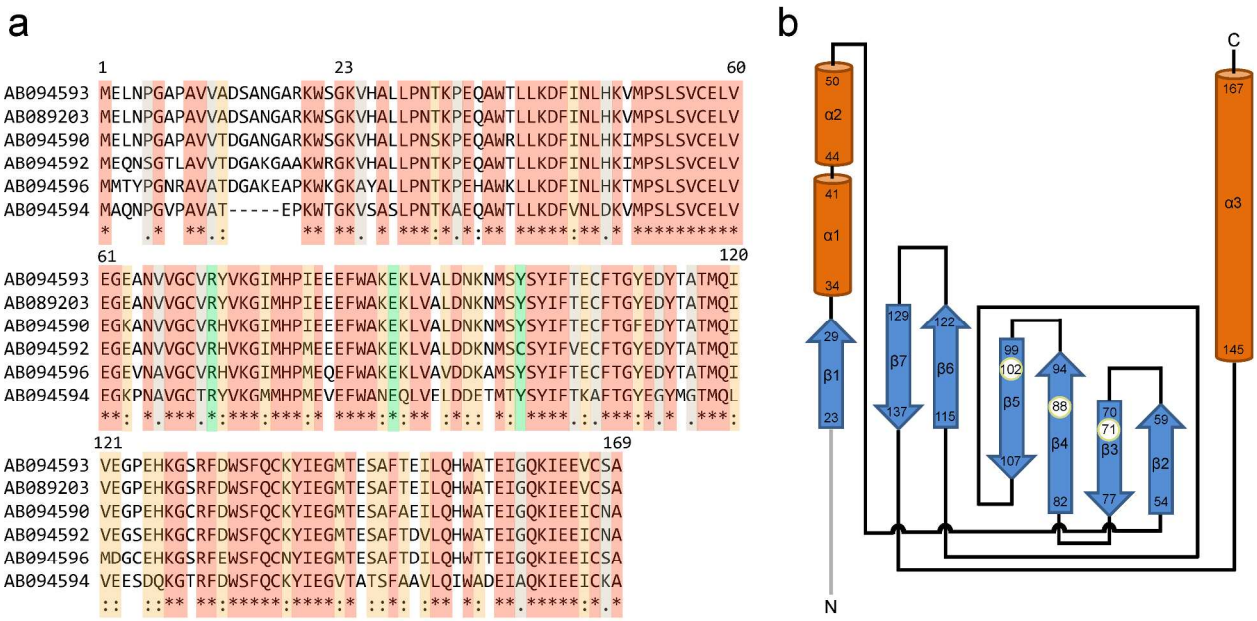
From the [1]Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH
[2]College of Wooster, Wooster, OH
[3]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY
[4]Northeastern Collaborative Access Team, Argonne National Laboratory, Argonne, IL
[5]Research Service, Louis Stokes Cleveland VA Medical Center, Cleveland, OH
[6]Cleveland Center for Membrane and Structural Biology, School of Medicine, Case Western Reserve University, Cleveland, OH

*To whom the correspondence should be addressed: Marcin Golczak, Ph.D., Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Ave, Cleveland, Ohio 44106, USA; Phone: 216–368–0302; Fax: 216–368–1300; E–mail: mxg149@case.edu.

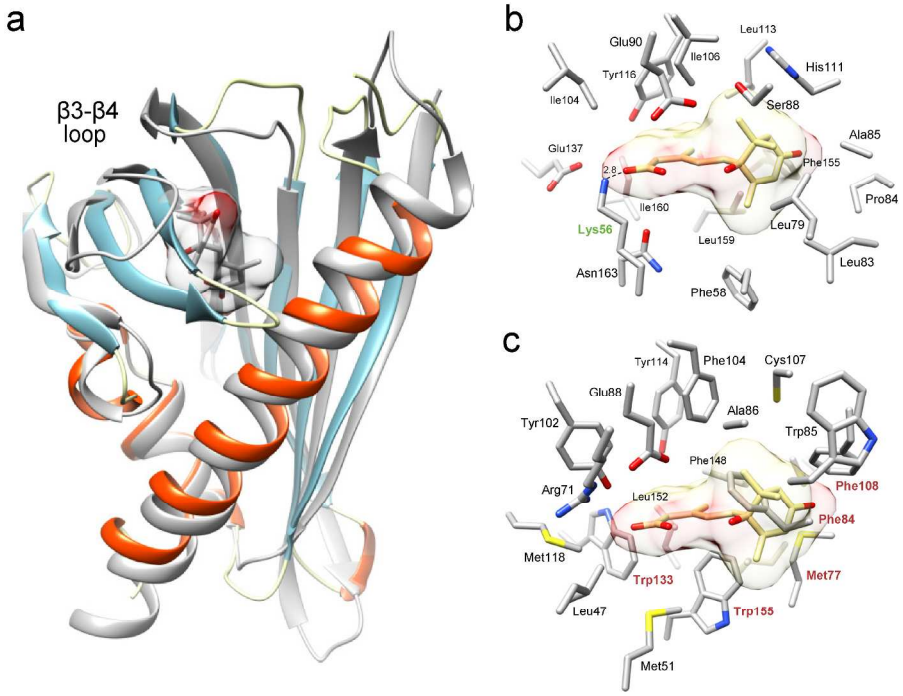
[#]these authors contributed equally to this work



Supplementary Figure 1 – *Purification of LFS.* (a), schematic representation of GST-fused LFS expressed in *E. coli*. To facilitate efficient thrombin cleavage of the fusion protein, PGISGGGG sequence was added to the N-terminus of LFS. (b), comparison of efficiency of thrombin digestion of GST-LFS proteins with and without PGISGGGG sequence. (c), SDS-PAGE of LFS after GST tag cleavage and final purification on HiTrap Q HP ion exchange column. The gel was stained with Coomassie Blue R-250. Fractions pooled together and used for the crystallization are marked in red. (d), typical crystals of LFS obtained in 0.1 M sodium acetate, pH 4.5, 30% (w/v) PEG 3350. Scale bar corresponds to 250 μm.



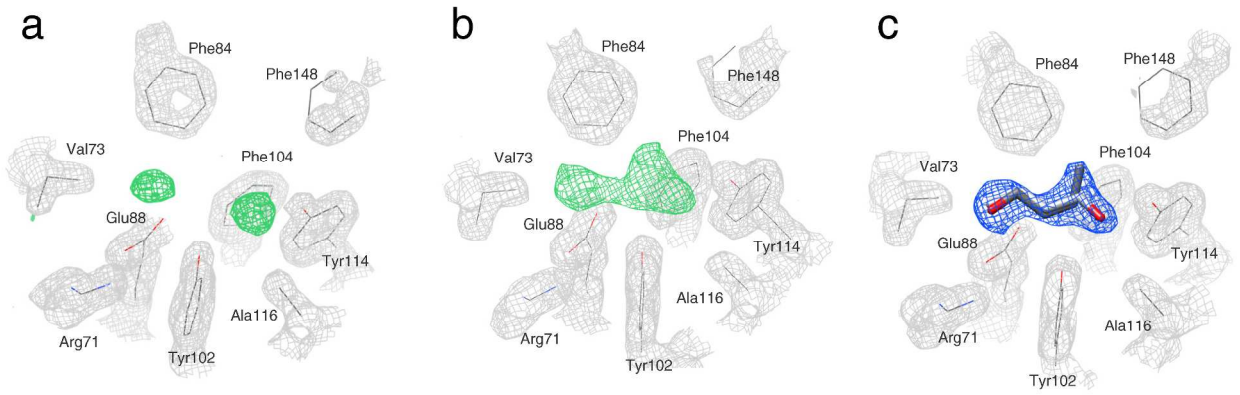
Supplementary Figure 2 – *Primary and secondary structure of LFS.* (a), sequence alignment of LFSs from selected *Allium* species. The NCBI accession numbers correspond to LFSs from the following plants: AB094593, *A. ascalonicum* (shallot); AB089203, *A. cepa* (onion); AB094590, *A. fistulosum* (Japanese bunching onion); AB094592, *A. chinense* (rakkyo); AB094596, *A. ampeloprasum* (elephant garlic); AB094594, *A. porrum* (leek). The sequences were aligned by using Clustal Omega available at the EMBL-EBI server. Residues involved in catalysis are colored green. (b), topology diagrams of onion LFS generated based on the crystal structure. Positions of residues involved in catalysis are indicated by white circles.



d

	PYL1	PYL2	PYL3	PYL5	PYL9	PYL10	PLY13	holo-LFS
apo-LFS	2.22	2.12	2.18	2.01	1.94	1.90	2.06	0.11

Supplementary Figure 3 – *Structural comparison of LFS and PYL10.* (a), structural comparison of LFS (colored based on the secondary structure blue, orange, and yellow) and PYL10 (PDB accession code 3R6P) depicted in gray. Although highly similar, position of β3 and β4 as well as a loop that connects these two β-strands revealed a major difference that contributes to the reduced size of the binding pocket in LFS. (b) and (c), consequence of altered position of β3-β4 strands on architecture of the intramolecular binding site. The orientations of side chains that form abscisic acid binding site in PYL10 (panel b*)* are contrasted with the position of analogue residues in LFS. Several large hydrophobic side chains (labeled in red) protrude into the space occupied by the ligand in PYL10. (d), r.m.s.d. values between LFS and various representatives of PYL protein family calculated for the main chain atoms.



Supplementary Figure 4 – *Interpretation of electron density maps in the vicinity of the ligand binding-site.* The green meshes symbolize an σA-weighted F_o - F_c omit maps (calculated after removing of the ligands and performing simulated annealing refinement) contoured at 3.6σ, whereas the grey meshes correspond to the $2F_o$ - F_c electron density contoured at 1.4σ. Panel (a) represents maps calculated for the structure of apo-protein. Panel (b) corresponds to electron density for the structure of holo-enzyme refined in the absence of the ligand. The appearance of a strong positive electron density clearly indicates the location of crotyl alcohol molecule. In the panel (b), the blue mesh signifies $2F_o$ - F_c electron density contoured at 1.2σ for structure of holo-LFS refined in the presence of crotyl alcohol at 57% and 43% occupancies for its *Z*- and *E*-isomers.